# Supporting Information

## Essere et al. 10.1073/pnas.1308649110

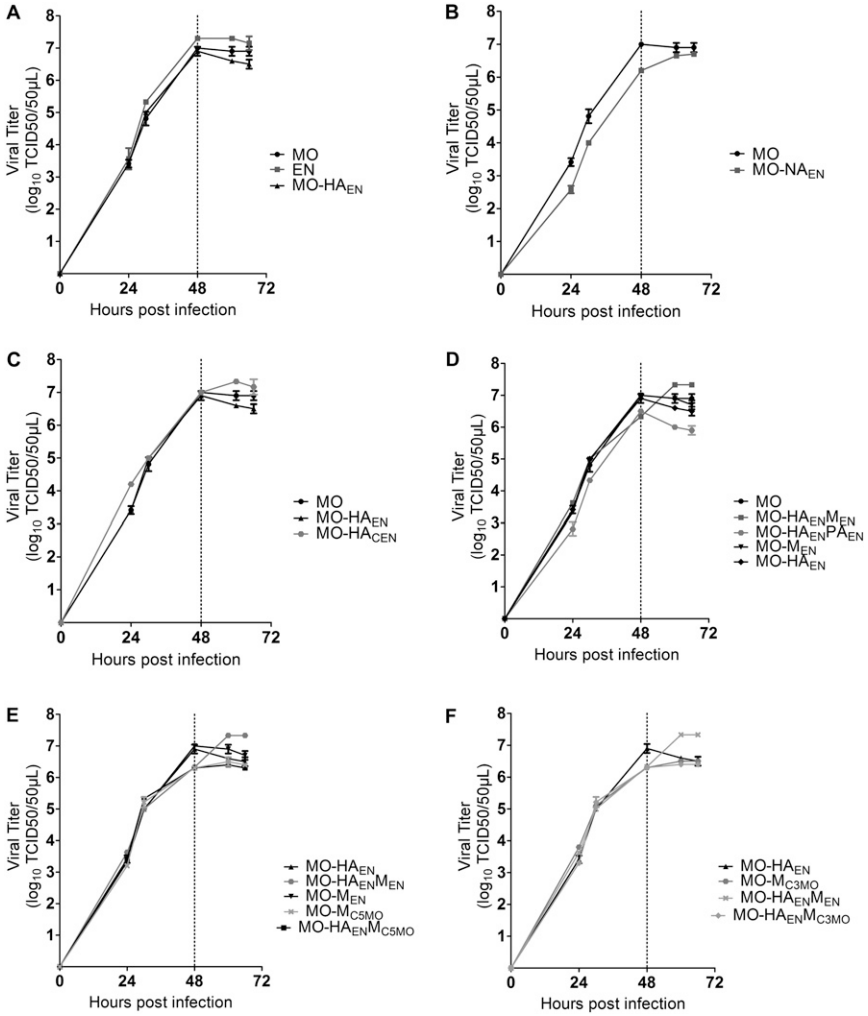


**Fig. S1.** Kinetics of replication of all recombinant viruses used in this study in Madin–Darby canine kidney (MDCK) cells (*A*–*F*). The genomic compositions of recombinant viruses was described in Table 3. MDCK cells were infected at a multiplicity of infection of $10^{-4}$ 50% tissue infective dose ($TCID_{50}$) per cell (1, 2). After a 1-h viral adsorption period, cells were overlaid with Eagle's minimum elementary medium (Lonza) supplemented with 1 μg/mL trypsin (Roche Diagnostics) and were incubated further at 37 °C. At 0, 24, 30, 48, 60, and 66 h postinfection, supernatants were harvested and centrifuged at 1,500 × *g* for 10 min and were stored at −70 °C. End-point titration assays were performed on confluent layers of MDCK cells in 96-well plates. Briefly, 50 μL of 10-fold serial dilutions of each virus were inoculated into four replicate wells. The 96-well microplates were incubated at 37 °C, and the presence of cytopathic effects was monitored 72 h later under the microscope. The $TCID_{50}$/50 μL values were determined using the Reed and Muench statistical method (2). Data points represent virus titer (mean ± SD) of $\log_{10}$ TCID50/50 μL.

1. Fournier E, et al. (2012) A supramolecular assembly formed by influenza A virus genomic RNA segments. *Nucleic Acids Res* 40(5):2197–2209.
2. Moulès V, et al. (2011) Importance of viral genomic composition in modulating glycoprotein content on the surface of influenza virus particles. *Virology* 414(1):51–62.

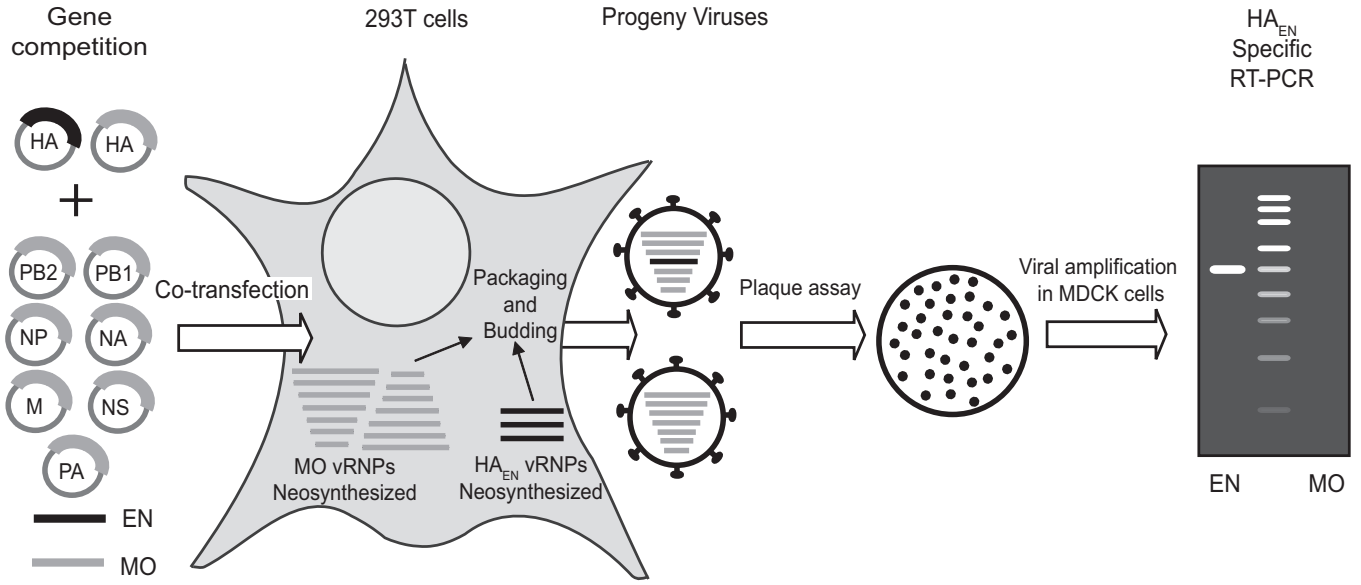


**Fig. S2.** Experimental strategy used for the rescue of reassortant virus using nine reverse genetic plasmids. 293T cells were cotransfected with the eight plasmids required for producing the A/Moscow/10/99 (H3N2) MO virus plus the plasmid corresponding to $HA_{EN}$. Equal amounts of the nine plasmids were used, except when specifically indicated in the main text. Viruses were harvested 48 h after transfection and were used for plaque assays on MDCK cells. Then viruses were amplified on MDCK cells to identify the origin of the HA gene by RT-PCR.

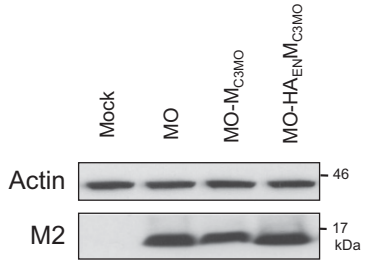


**Fig. S3.** M2 protein levels in influenza-infected MDCK cells. MDCK cells were mock infected or were infected by MO, MO-$M_{C3MO}$, and MO-$HA_{EN}M_{C3MO}$ at a multiplicity of infection of $10^{-3}$, and total protein lysates were harvested 48 h postinfection. Thirty micrograms of total protein per condition were analyzed by Western blot using specific antibodies for actin (ab8227; Abcam), and M2 (sc-32238; Santa Cruz). Actin was used as a loading control. These results indicate that the nucleotide changes in the $M_{C3MO}$ chimera, notably in the main 5′ splice site (position 55), do not affect the M2 protein levels.

**Table S1. Primers used for site-directed mutagenesis on the plasmid containing the cDNA from the human H3N2 M gene**

| | Forward primer | Reverse primer | Temperature (°C) |
|---|---|---|---|
| 1 | GGTAGATATTGAAAGATGAGCCTTCTAACCGAGGTCG | CGACCTCGGTTAGAAGGCTCATCTTTCAATATCTACC | 79.4 |
| 2 | CCGAGGTCGAAACGTATGTTCTCTCTATCGTC | GACGATAGAGAGAACATACGTTTCGACCTCGG | 78.0 |
| 3 | GTTCTCTCTATCGTTCCATCAGGCCCCCTCAAAGC | GCTTTGAGGGGGCCTGATGGAACGATAGAGAGAAC | 78.8 |
| 4 | CCCCCTCAAAGCCGAAATCGCGCAGAGACTTG | CAAGTCTCTGCGCGATTTCGGCTTTGAGGGGG | 81.6 |

**Table S2. Primers used to determind gene origin**

| Viral gene | Gene origin | Restriction site | Forward primer | Reverse primer |
|---|---|---|---|---|
| HA | EN | —— | CCGTTGGAACATCAACACTG | CTTTGTCTGCAGCGTATCCA |
| PB1 | MO | —— | TAAGGCCTCTTCTAATAGATGG | AAGTCTTCCCCGATAATCC |
| NP | EN | —— | GGTCCAATCTACCGAAGGAG | TGTTTTGAAGCAGACGGAAA |
| NA | MO | —— | CTGACCAACACCACCATAGAGA | AAGCAATGGCTACTGCTGG |
| NS | EN | —— | GTAGACCAAGAACTGAGTGATGCC | CTGATGCAAAGAGGTCCTTCC |
| M | EN | MunI | GACTGCAGCGTAGACGCTTTG | CGATATTCTTCCCTCATAGACTCAGG |
| PA | EN | NcoI | CAACAAGGCATGCGAACTGAC | CAAAGAATTCTTTGGTCATGTCTTTCTC |
| PB2 | MO | NcoI | GCTGTGGATATATGCAAGGCTG | TCTCCCACATCATTGACGATG |

PCR amplification with specific primers was performed for all viral genes to identify gene origin. For M, PA, and PB2 genes, the analysis of restriction enzyme profiles (MunI or NcoI) was necessary to confirm the gene origin. EN, H5N2 A/Finch/England/2051/91; MO, H3N2 A/Moscow/10/99.